IN THE CLAIMS

Please cancel claim 19 without prejudice.

REMARKS

Applicant has amended the specification to insert SEQ ID NOS. on pages 6, 7, 9 and 10 and has replaced Sequence Listing pages 1-13, filed April 16, 2001 with new Sequence Listing pages 1-14 submitted herewith.

Applicant has canceled claim 19 without prejudice. Applicant expressly reserves the right to prosecute the subject matter of the canceled claim in one or more further applications that claim priority under 35 U.S.C. § 120 from this application. Upon entry of the amendments, claims 1-5, 7, 9, 11 and 15-18 will be pending in the application. Claims 1-3 and 5 remain pending but are withdrawn from examination as drawn to a non-elected invention.

Applicant will discuss the amendments in detail in connection with the Examiner's rejections. None of the amendments introduces new matter. Applicant requests entry of the amendments and reconsideration of the pending claims.

Sequence Listing Rules

The Examiner states that the above-identified application is still not in complete compliance with the sequence rules. The Examiner states that the above-identified application does not comply with 37 C.F.R. 1.821(d) which requires that all sequences embedded in the specification be accompanied by the appropriate sequence identifier.

Accordingly, applicant has amended the specification at pages 6, 7, 9 and 10 to include sequence identifiers with all sequences embedded in the specification. Applicant also

submits marked-up copies of the specification pages 6, 7, 9 and 10 entitled "Version with markings to show changes made" showing changes that have been made. The underlining marking on these marked-up copies indicates addition of text. Furthermore, applicant has replaced Sequence Listing pages 1-13, filed April 16, 2001, with new Sequence Listing pages 1-14. Applicant encloses herewith the substitute Sequence Listing pages 1-14, a Computer Readable Form submission of same and the required Statements.

Objection Under 37 C.F.R. § 1.75(c)

The Examiner has objected to claim 19 under 37 C.F.R. § 1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Furthermore, the Examiner contends that claim 19 fails to further limit the subject matter of claim 16, insofar as claim 16 depends from claim 18, which recites a subunit of a virus, and claim 16 is drawn to an embodiment reciting a vaccine additionally comprising a subunit of a virus.

This objection has been rendered moot by the cancellation of claim 19 herein.

35 U.S.C. § 112, Second Paragraph

Claims 4, 7, 9, 11, 15-19

Claims 4, 7, 9, 11 and 15-19 stand rejected under 35 U.S.C. § 112, second paragraph, as "indefinite" for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

With respect to claims 4, 11 and 16, the Examiner alleges that the term "homologous sequences having at least 75% homology to SEQ ID NO:..." is indefinite essentially for reasons of record in rejecting the claims in the previous

Office Action. The Examiner states that applicant's previous arguments have been considered and not found persuasive.

In particular, with respect to claim 16, which recites "homologous" in the context of a non-coding region of nucleotide sequence, the Examiner states that applicant's argument as directed to amino acid sequence is not understood and is not seen to apply. Furthermore, the Examiner states that with respect to claim 4, which recites "homologous sequences having at least 75% homology to the SEQ ID NO:4," taking into account applicant's apparent reliance on the default parameters of the BLITZ software as a definition of "75% homology," it is still not apparent what combination of identical and "similar" amino acids is being claimed, and it is unclear what type of similarity is required in order to be encompassed by the claims. Moreover, the Examiner states that neither homology nor similarity is described or defined in the specification in such a way that one of ordinary skill in the art can unambiguously determine the metes and bounds of the claims (Office Action, p. 3, lines 4-22; p. 4, lines 1-3). Applicant respectfully traverses.

To address the examiner's rejection of claim 16, which recites "homologous" in the context of a non-coding region of nucleotide sequence, applicant respectfully submits that the specification teaches that homology was determined by comparing the nucleotide sequences of the virus isolates upon alignment of the sequences as illustrated in Table 2. These nucleotide sequences were generated by first producing an initial fragment of a 303 bp PCR product for the three Ljungan virus isolates 87-012, 174F and 145SL using cardiovirus consensus primers. This was followed by an alignment of the 5'UTR sequences of the 303 bp PCR product for each of the three Ljungan isolates, as displayed in Table 2. Based on the

alignment, the homology or homologous sequences between different sequences could then be determined.
Sequence homology was then determined between the three Ljungan isolates as well as between published cardioviruses (e.g., TMEBeAn, Vilyuisk, and EMCV). The alignment starts 29 nt 3' of the end of the poly-C tract in EMCV and the sequence corresponds to nt 557-808 (approximately) in the different viral genomes. Inserted spaces in the alignment are indicated by a period. Based on this analysis, applicant states that the Ljungan 174F has a 94% homology to Ljungan 87-012 (here taken as the indicator strain for comparisons) and Ljungan 145SL has 91% homologous residues to Ljungan 87-012. Thus, one skilled in the art would understand what is meant by "homologous sequence" or "homologous" in the claim 16 (and claim 11).

To address the examiner's rejection of claim 4, which recites "homologous sequences having at least 75% homology to the SEQ ID NO: 4", applicant respectfully submits that the specification teaches that an additional PCR fragment from the Ljungan virus isolate was obtained by amplifying the cDNA using the SLJU1 or SLJU2 primers in combination with the 118 10-mer oligonucleotide, which yielded a 1.8-1.9 kb PCR product. Of this fragment, 819 bp were sequenced from the 3' end. This sequence was found to contain an open reading frame (ORF) of 663 bp in the sense of the viral polyprotein. This ORF was then used to search the Swiss protein data bank using the BLITZ search service from EMBL with the default search parameters. The top ten scores were picornavirus polyprotein sequences, including 8 cardiovirus sequences. Homology was found over 188 amino acids and the relatedness of this 188 amino acid segment of the viral polyprotein was aligned with previously sequenced cardioviruses as shown in Table 3. The

alignment in Table 3 uses the TMEBeAn was taken as the index strain and the remaining 12 cardiovirus sequences and Ljungan 145SL sequence were compared with the index strain, with only the differences in amino acid sequence being shown. Since the BLITZ search algorithm takes into account identical as well as similar amino acids, the latter have been indicated by small type, while differences to TMEBeAn is in capitals as for the other strains in the alignment. While the amino acid homology (identical amino acids) of the viruses within the Theiler group is 96-97%, the homology to Vilyuisk virus is about 83%, and the EMC viruses are 67-74% homologous to the TMEBeAn, the Ljungan 145SL has only 32% identical amino acids to TMEBeAn. Even if homology is taken as identical and similar amino acids, this measure of relationship would still amount to only 50% between Ljungan 145SL and TMEBeAn (the corresponding figure would be 79-83% between EMC and TMEBeAn). Thus, the Ljungan viruses are related to the cardioviruses, when the sequences from the highly conserved part of the 5'UTR were compared, but the Ljungan viruses are clearly more distant relatives than any previously identified cardiovirus.

The BLITZ search algorithms base the search on homology and similarity between the unknown sequence of interest and known sequences present in the database. The parameters which govern such a search can be set at default levels or adjusted according to what information one would like to obtain from the search results. Thus, one of skill in the art would know how to adjust the parameters to derive at the type of search result desired. Furthermore, it is well-known in the art that similar amino acids refers to amino acids that are physically or functionally similar to the corresponding reference amino acid. For example, the amino acids may have similar size, shape, electric charge, chemical

properties including the ability to form covalent or hydrogen bonds or the like. Preferred conservative substitutions are those fulfilling the criteria defined for an accepted point mutation in Dayhoff et al., Atlas of Protein Sequence and Structure, 5, pp. 345-352 (1978 & Supp.). Examples of similar amino acids include but are not limited to the following groups: (a) valine, glycine; (b) glycine, alanine; (c) valine, isoleucine, leucine; (d) aspartic acid, glutamic acid; (e) asparagine, glutamine; (f) serine, threonine; (g) lysine, arginine, methionine; and (h) phenylalanine, tyrosine. Thus, one of skill in the art would be able to determine what the metes and bounds contained using the terms homology and similarity. Applicant respectfully request withdrawal of this rejection.

Claim 17

The Examiner has rejected claim 17 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Specifically, the Examiner contends that claim 17 is confusing as it depends from claim 4 and recites "comprising an antigen" since it is not clear whether the antigen is the same as the "antigenic fragment" of claim 4 or some additional, undefined component. Applicant respectfully traverses.

Applicant submits that the recitation "comprising an antigen" in claim 17 is different from the element "antigenic fragment" in claim 4. Claim 4 recites a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4 and homologous sequences having at least 75% homology to the SEQ ID NO: 4, and antigenic fragments of the sequences. Claim 17 recites a protein according to claim 4, comprising an antigen. The terms "antigenic fragments" and "antigen" refer to different elements. The application as filed describes a

method for determining if a protein of the invention or a fragment thereof is an antigen (see, e.g., page 4, line 30 to page 5, line 14). Applicant respectfully request withdrawal of this rejection.

Claims 7, 11, 15 and 16

The Examiner has rejected claims 7, 11, 15 and 16 under 35 U.S.C. § 112, second paragraph, for allegedly being indefinite. Specifically, the Examiner contends that claims 7, 11, 15 and 16 are confusing because each depends alternatively from claims 4 and 17 and recites "an antibody-binding part" of a protein and it is not clear whether the cited material is the same as the "antigenic fragment" of claim 4 or the "antigen" of claim 17. Applicant respectfully traverses.

Applicant submits that the recitation "an antibody-binding part" of a protein in claims 7, 11, 15 and 16 is different from the elements of "antigenic fragment" of claim 4 or the "antigen" of claim 17. The terms "antibody-binding part", "antigenic fragments" and "antigen" all refer to different elements. The term "an antibody-binding part" of a protein refers to the specific structural part of a protein which is recognized by an antibody such that the antibody can bind to that structural part of the protein. On the other hand, an "antigen" and "antigenic fragment" includes a protein or a fragment thereof which can elicit an immune response. Thus, an "antibody-binding part" of a protein recited in claims 7, 11, 15 and 16 is not the same as the "antigenic fragment" of claim 4 or the "antigen" of claim 17. Applicant respectfully request withdrawal of this rejection.

35 U.S.C. § 112, First Paragraph

Claims 4, 7, 9 ,11 and 15-19

Claims 4, 7, 9 ,11 and 15-19 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly not enabling. Specifically, the Examiner asserts that the specification, while enabling for a protein SEQ ID NO: 4 does not enable a protein having at least 75% homology to SEQ ID NO: 4, or an antigenic or antibody-binding portion of SEQ ID NO:4, nor an antigenic or antibody-binding portion of a protein having at least 75% homology to SEQ ID NO: 4, nor a viral subunit essentially for the reasons of record in rejecting claims 4, 6 and 7 in the previous Office Action. The Examiner states that applicant's previous argument that since the specification as filed refers to at least one algorithm (BLITZ) for determining amino acid sequence homology, one of ordinary skill in the art would readily be able to make and use proteins at least 75% homologous in amino acid sequence to SEQ ID NO: 4 without undue experimentation has been considered but not found persuasive. Applicant respectfully traverses.

As applicant has discussed earlier, the specification recites the use of the BLITZ search algorithm which compares sequences based on homology and similarity. Accordingly one of skill in the art would be able to determine homologous (identical) and similar sequences when using the BLITZ search program and would readily be able to make and use proteins at least 75% homologous in amino acid sequence to SEQ ID NO: 4 without undue experimentation.

Furthermore, the Examiner asserts that there is no guidance as to which of the myriads of possible sequences and proteins included would function as required in order that one of skill in the art would know how to use all the proteins encompassed by the claims. Applicant respectfully traverses. The specification states that the sequences be at least 75% homologous in amino acid sequence to SEQ ID NO: 4 and provides

adequate enablement for one of skill in the art to determine this homology using the BLITZ algorithm program.

The Examiner also states that applicant's previous argument that the amended claims now recite a protein comprising an antigen and that the application as filed teaches a method for determining if a protein or a fragment is antigenic and cites specification p. 4, line 30 to p. 5, line 14, as describing such a method has been considered but not found persuasive. Specifically, the Examiner asserts that the specification describes immunization of mice with a cell culture supernatent and subsequent indirect immunofluorescence test using a preparation of infected cells to detect antibodies produced by the immunization and does not teach how to determine antigenic fragments of a protein comprising SEQ ID NO: 4 or sequences homologous to SEQ ID NO:4. Applicant respectfully traverse.

The specification teaches that viruses can be isolated by inoculating saliva/lung homogenates or faeces into BHK-21 cells and at the earliest signs of cytopathogenic defect, cells were removed and placed on slides for staining with a panel of human sera from patients using immunofluorescence test (IFT). Cells which demonstrated a positive reaction by IFT were subsequently injected into animals intraperitoneally (cell culture supernatant from BHK-21 cells) and serum was collected 4-6 weeks later in order to raise antisera to the virus isolates in the immunized animals. The serum was then subjected to IFT or Plaque reduction neutralization test (PRNT) to determine the antibody titers in the serum.

One of ordinary skill in the art would know that a positive interaction between a virus and immunogenic sera must involve an antigenic fragment on the virus since antigens are

molecules that can bind to a specific antibody. Thus, in order for the human sera from patients to positively react with the virus-infected cells, the cells must be expressing an antigenic fragment of the virus recognized by the antibodies in the human sera. Similarly, the ability to induce the generation of antibodies upon injection of the culture supernatant from virus-infected cells into animals suggests the presentation of antigenic fragments by the virus. Furthermore, the particular antigenic fragments of the virus-infected cells would be highly homologous to the SEQ ID NOs disclosed since these virus-infected cells were subsequently sequenced and homology of the isolated virus was compared with other known viruses by using the BLITZ search algorithm program.

Thus, the application as filed does teach how to determine antigenic fragments of proteins comprising SEQ ID NO: 4 or sequences homologous to SEQ ID NO: 4. Accordingly, claims 4, 7, 9, 11 and 15-19 are fully enabled by the application, as filed.

Claims 15, 18, 9, 11, 16 and 19

Claims 15, 18, 9, 11, 16 and 19 stand rejected under 35 U.S.C. §§ 112, first paragraph, as allegedly nonenabling. The Examiner alleges that claims 15, 18, 9, 11, 16 and 19 are rejected for essentially the same reasons of record in rejecting claims 9-12 and 14-16 in the previous Office Action. Specifically, the Examiner asserts that the claims are drawn to pharmaceutical compositions, vaccines and methods of use. Furthermore, the Examiner states that applicant's previous arguments that the application as filed provides factual evidence of human infection by a virus of the invention and points to p. 14, lines 1-28, describing human sera containing

antibodies specific for "the viral proteins of the invention," as well as to p. 3, lines 4-11, describing similar viruses, and has cited Jun et al., Dan et al., and Tolbert et al., all cited as describing murine infections with picornaviruses that are associated with diseases or pathological conditions have been considered but found not persuasive. According to the Examiner, the specification at p. 14, lines 1-28, disclose that antibodies present in human sera from patients disclosed as having diabetes mellitus or myocarditis immunoreact with certain viral-infected African green monkey kidney cells. Furthermore, the Examiner states that it is not even apparent that the antibodies detected are specific for the protein that is recited in the instant claims, and there is no evidence that any disease is actually caused by the Ljungan virus. Furthermore, the Examiner states that there is no factual evidence that would indicate that administration of the protein that comprises the amino acid of SEQ ID NO: 4 and/or a subunit of the virus from which the recited sequences originate would have any beneficial effect for any mammal, including humans, although such use is encompassed by the claims. Moreover, the Examiner contends that the documents cited by applicant do not disclose any information concerning the Ljungan virus but rather are concerned with other, different picornaviruses. Applicant respectfully traverses.

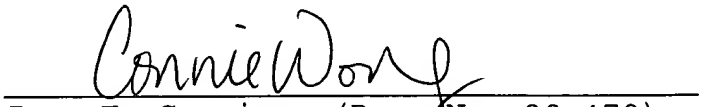
The specification discloses the isolation of three different virus isolates which exhibit cytopathic effect (CPE) and react with a large panel of human sera using immunofluorescence test (IFT). Specifically, the panel of human sera tested included 5 multiple sclerosis patients, 5 patients recently diagnosed with Diabetes Mellitus and 5 athletes dying in myocarditis. Only cells positively reacting with the human serum panels were selected for further

analysis. The three virus isolates disclosed all displayed sizes and structures compatible with a picornavirus based on electron microscopy. Moreover, these three virus isolates were able to kill suckling mice within 3-5 days when inoculated intracerebrally into 1-day old suckling mice. Alignment of the sequences from the three virus isolates with published cardiovirus sequences demonstrated that there was substantial homology both at the nucleotide and amino acid levels with the known cardiovirus sequences. Furthermore, sera were collected from patients diagnosed with diabetes mellitus or myocarditis reacted positively with Green Monkey Kidney cells infected with the three virus isolates as demonstrated by IFT. Of these serological tests, serum from 32% of the children with Diabetes Mellitus reacted positively to one or more of the three viruses (vs. 6% for controls) while serum from 56% of adults with Diabetes Mellitus reacted positively to one or more of the three viruses (vs. 6% for controls). Similarly, serum from 80% of patients dying from myocarditis reacted positively to one or more of the three viruses (vs. 7% for controls). Thus, there is statistical evidence to support that the virus isolates disclosed in the present invention are involved in pathological diseases such as Diabetes Mellitus and myocarditis. Accordingly, applicant requests that the rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

CONCLUSION

Applicant believe that in view of the foregoing, the claims are in condition for allowance. Accordingly, applicant requests that the Examiner enter the amendments presented herein, consider the foregoing remarks and allow the pending claims to issue.

Respectfully submitted,



Jane T. Gunnison (Reg. No. 38,479)
Attorney for Applicant
Connie Wong (Limited Recognition)
Agent for Applicant
c/o FISH & NEAVE
1251 Avenue of the Americas
New York, New York 10020-1104
Tel.: (212) 596-9000

Electron micr sc py

Cell culture media or brain tissue homogenates were examined by negative contrast electron microscopy (EM). A 10 µl droplet was incubated on Formvar/carbon-coated grids for one minute or alternatively, 0.5 ml samples were centrifuged for 30 minutes at 20,000 x g to remove cell debris and finally the supernatants were pelleted directly onto grids in a Beckman Airfuge for 10 minutes at 160,000 x g. Grids were stained with 2% phosphotungstate acid (pH 6.0) and examined in a Philips CM 100 electron microscope at a magnification of at least 46,000.

Sequence data

The isolates 87-012, 174F and 145SL were grown on the human lung carcinoma line A549 in 1600 cm^2 roller bottles. Full CPE was observed after 5-10 days. Supernate was filtered through 0.45 µM cellulose acetate filters (Costar) and the virus was pelleted at 20,000 g for 20 h at 4°C. RNA was isolated from the virus containing pellets using acid guanidinium thiocyanate as described (Chomczynski and Sacchi). Synthesis of cDNA was performed under standard conditions using 1 µg of RNA, AMV reverse transcriptase (Boehringer-Mannheim) and random 14 mer oligonucleotides as primers in a 20 µL reaction. Fragments of the viral 5'UTR were amplified using cardiovirus specific consensus primers: (sense) 5'-GGCCGAAGCCGCTTGGAATA-3' (SEM) [SEQ ID NO. 21] and (antisense) 5'-GTGGCTTTTGGCCGCAGAG-3' (ATVEM), [SEQ ID NO. 22] both primers modified after the EMCV2 and EMCV1 primers previously reported (Jongen et al. 1993. Ann. Reum. Dis. 52:575-578. Cardiovirus sequences were from Dr A. Palmenberg (personal communication). Amplification conditions were 30 cycles at: 94°C, 30 sec., 50°C, 30 sec, 72°C, 2 min. The amplified fragments were cloned into the pCRII T-vector (In-Vitrogen). The cloned viral sequences were sequenced using A Taq polymerase FS cycle sequencing kit and data was collected on a ABI Prism 310 sequencing machine using M13 -21 and M13 reverse primers (Perkin-Elmer). A 1.8 kb fragment extending from the 5'-UTR into the viral polyprotein sequences was obtained by PCR (polymerase chain reaction) amplification of cDNA from the 145SL isolate. The primers were:

(sense) 5'-ACAGTGCATTCCACAC-3' (SLJU1) [SEQ ID NO. 23] or 5'-CCGCTCCACAATAGA-3' (SLJU2) [SEQ ID NO. 24] and (antisense) 5'-GATCTCAGAC-3' (primer 118) [SEQ ID NO. 25]. The SLJU1 and SLJU2 primers are located immediately adjacent to one another and were chosen as consensus primers for the Ljungan isolates of the invention with as little homology as possible to the EMCV and TMEV groups of viruses. The amplification conditions were 30 cycles at: 94°C, 30 sec., 42°C, 1 min , 72°C 2 min. The antisense primer 118 [SEQ ID NO. 25] yielded similarly sized PCR products with either the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] as sense primers, but none of the primers yielded PCR fragments when used alone. The sequence of the primer 118 [SEQ ID NO. 25] was previously published (Bauer, D., et al. 1993. Nucl. Acids Res. 21:4272-4280). The obtain d 1.8 kb PCR fragment was cloned and sequenced as described above.

RESULTS OF EXPERIMENTAL WORK

Three virus isolates were selected based on reaction with the human serum panels and showing a size and structure compatible with a picornavirus on EM. The first isolate was named Ljungan 87-012. Ljungan is a river in Medelpad county, Sweden where the animals were trapped.

The second and third isolate were designated Ljungan 174F and Ljungan 145SL, respectively.

All three isolates came from C. glareolus.

All three isolates killed suckling mice in 3-5 days.

The titer in mouse brain was 10^9 (approximately) while the cell culture titer was only 10^5 (approximately).

Electron microscopy

Virus particles, 27 nm in diameter, were spherical with the surface almost featureless and they appeared single or in small aggregates. In rare cases the stain penetrated the particles which made them look like empty shells.

obtaining products from EMC virus cDNA. The subsequent sequence analysis revealed that the ATVEM primer was mismatched at 4 internal positions, explaining this difference in reannealing temperature. An alignment of the 5'UTR sequences for the three Ljungan isolates, EMCV and Vilyuisk virus (Table 2) shows a greater similarity between EMCV and Vilyuisk virus than between either of the two and the Ljungan isolates. It also demonstrates that each Ljungan isolate is distinct from the other by a number of nucleotide changes. The 174F and 145SL are similar to the isolate 87-012. The sequence homology between 174F and 87-012 was at most 95% (three undetermined bases in the sequence) while the homology between 87-012 and 145SL was 91%.

The strategy chosen for obtaining additional PCR fragments from the Ljungan virus isolates was a modification of a technique for detecting differentially expressed mRNAs (Bauer, D., et al. 1993. Nucl. Acids Res. 21:4272-4280). As a test for this strategy, cDNA from the Ljungan 145SL isolate was amplified using the conditions above, using either the SLJU1 [SEQ ID NO. 23] or the SLJU2 [SEQ ID NO. 24] primer as a sense primer and one of twenty 10-mer oligonucleotides of randomly chosen sequence as "antisense" primer.

If the PCR products obtained with the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] primers and a specific 10-mer were similarly sized, and none of the primers yielded a product of this size when used alone in the PCR reaction, the fragment obtained was isolated and cloned. Only one combination of primers satisfied this criterion, namely the SLJU1 [SEQ ID NO. 23] or SLJU2 [SEQ ID NO. 24] primers in combination with the 118 10-mer oligonucleotide [SEQ ID NO. 25], which yielded a 1.8-1.9 kb PCR product. Of this fragment, 819 bp were sequenced from the 3' end. This sequence contained an open reading fram (ORF) of 663 bp in the sense of the viral polyprotein. This ORF was used to search in the Swiss protein data bank using the BLITZ search service from EMBL with the default search parameters. The top 10 scores were picornavirus polyprotein sequences, including 8 cardiovirus sequences. Homology was found over 188 a.a. The relatedness of this segment of the viral polyprotein to previously sequenced cardioviruses is shown in Table 3. A comparative

alignment of all cardioviruses was made available to us by Dr. A. Palmenberg. In Table 3, the sequence of TMEBeAn was arbitrarily taken as the index strain. For the 12 remaining cardioviruses in the alignment, only differences in amino acid sequence are shown. The alignment of the Ljungan 145SL sequence is similarly represented at the top. Since the BLITZ search algorithm takes into account identical as well as similar amino acids, the latter have been indicated by small type, while differences to TMEBeAn is in capitals as for the other strains in the alignment.

In conclusion, the above presented data for the Ljungan isolates are characteristic for the 3 viruses but yet incomplete. However, the comparison of cloned sequences from both a highly conserved part of the 5'-untranslated region of cardioviruses and coding sequences for the viral capsid proteins of one isolate (Ljungan 145SL) clearly show that the Ljungan viruses are related to the cardioviruses, but are more distant relatives than any previously identified cardiovirus. While the amino acid homology (identical amino acids) of the viruses within the Theiler group is 96-97%, the homology to Vilyuisk virus is about 83%, and the EMC viruses are 67-74% homologous to TMEBeAn, the Ljungan 145SL has only about 32% identical amino acids to TMEBeAn. Even if homology is taken as identical and similar amino acids, this measure of relationship would still amount to only 50% between Ljungan 145SL and TMEBeAn (the corresponding figure would be 79-83% between EMC and TMEBeAn).

ALIGNMENT OF SEQUENCES

Table 2 shows an alignment of three Ljungan virus isolates (1. 87-012, 2. 174F, 3. 145SL)[SEQ ID NO: 1,2 and 3, respectively] with published cardiovirus sequences (4. TMEBeAn, 5. Vilyuisk, 6. EMCV)[SEQ ID NO. 5, 6 and 7 respectively]. The aligned sequence starts 29 nt 3' of the end of the poly-C tract in EMCV, and the sequence corresponds to nt 557 - 808 (approximately) in the different viral genomes. Inserted spaces in the sequences are indicated by a period (.).